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(54) Title: SECRETION SIGNAL VECTORS

(57) Abstract: The present invention provides delivery vectors for transferring a nucleic acid sequence to a cell *in vitro*, *ex vivo* or *in vivo*. The delivery vector comprises a segment encoding a secretory signal peptide. In embodiments of the invention, the delivery vector is an adeno-associated virus (AAV) vector. In other embodiments, the secretory signal peptide is a fibronectin secretory signal peptide (including variations and modifications, thereof). The delivery vectors of the invention may further comprise a heterologous nucleic acid sequence encoding a polypeptide of interest for transfer to a target cell, where the polypeptide of interest is operably associated with the secretory signal. Also disclosed are methods of transferring a nucleic acid of interest to a cell using the delivery vectors of the invention.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/13228

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C12N 15/74; C07H 21/04;
US CL : 435/320.1; 536/23.4, 23.5
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/320.1; 536/23.4, 23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN/WEST/STIC SEQUENCE SEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 5342762 A (MOSHER et al) 30 August 1994, see abstract; column 3, lines 1-8; column 5, lines 41-48; claim 1; column 6, lines 24-29, and 50-55.	1,2,5,6,9,15 --- 1-9, 11, 13, 15-17
Y	PATEL et al , Organization of fibronectin gene provides evidence for exon shuffling during evolution, EMBO J. , 1987, Vol. 6, No. 9, pages 2565-2572, see entire document.	1, 2, 5, 6, 9, 15
X	YURCHENKO et al, Recombinant Laminin G Domain Mediates Myoblast Adhesion and Heparin Binding, 15 April 1993, J. Biol.Chem. Vol.268, No. 1, Pages 8356-8365, see entire document.	1, 2,5, 6,15
Y	Database Medline on STN, No 96414664, D'ERCOLE et al, 'Human insulin-like growth factor binding protein-1 (hIGFBP-1 transgenic mice', abstract, Progress in Growth Factor Research, 1995.	1,2,5-9,1 1,13,15
Y	WO 92/15015 A1 (MCKNIGHT et al) 3 September 1992, see page 3, lines 23-29, Fig. 1, page 6, lines 19-29; page 46, line 15 to page 48, line 5, and page 12, lines 21-33.	1, 2, 5-9, 1, 13, 15

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"B" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

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C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SCHWARZBAUER et al, Identification of the Fibronectin Sequences Required for Assembly of a Fibrillar Matrix, J. Cell. Biol. June 1991, Vol. 113, No. 6, pages 1463-1473, see page 1464, column 1, lines 4-7 of first full paragraph.	1,2,5,6,15
Y	GenBank Accession No. NM_002026, 15 November 2001, see entire document.	1, 6
Y	GenBank Accession No. Q91740, 15 July 1999, see entire document.	1, 6
Y	DESIMONE et al, Identification and Characterization of Alternatively Spliced Fibronectin mRNAs Expressed in Early Xenopus Embryos, Dev. Biol., 1992, Vol. 2, pages 357-369, see entire document.	1, 6
Y	US 5,618,677 A (NI et al) 8 April, 1997, see paragraphs 61, 63, and 64.	1-4, 15-17
Y	SAMULSKI et al, Helper-Free Stocks of Recombinant Adeno-Associated Viruses, J. Virol., September 1989, Vol. 63, No. 9, pages 3822-3828, see entire document.	1-4, 15-17
Y	US 6,365,394 B1, 2 April 2002, see paragraphs 35 and 36.	1, 7, 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/13228

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-9,11,13 and 15-17

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

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INTERNATIONAL SEARCH REPORT

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

Group 1 claim(s) 1-14, partially, drawn to delivery vectors comprising a heterologous chimeric nucleic acid sequence comprising (i) a segment encoding a polypeptide of interest, and (ii) a segment encoding a fibronectin secretory signal that is operatively associated therewith, said heterologous chimeric nucleic acid sequence encoding a fusion polypeptide.

Group 2, claim(s) 18-20, and 24-31, drawn to methods of delivering a vector to a cell of the central nervous system.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack unity of invention because they are not so linked as to form a single general inventive concept under PCT Rule 13.1.

In order for more than one species to be examined, the appropriate additional examination fees must be paid. The species are as follows:

Fibronectin secretory signals selected from the group consisting of
1) SEQ ID NO:2 and SEQ ID NO:4, fragments thereof of at least 15 amino acids, signal encoded by a nucleic acid that hybridizes to a nucleic acid encoding SEQ ID NO: 2, SEQ ID NO:4 or a fragment thereof of at least 15 amino acids, and secretory signals that are at least about 80% identical to SEQ ID NO: 2, SEQ ID NO:4 or a fragment thereof of at least 15 amino acids,

2) -SEQ ID NO:5, fragments thereof of at least 15 amino acids, signal encoded by a nucleic acid that hybridizes to a nucleic acid encoding SEQ ID NO: 5 or a fragment thereof of at least 15 amino acids, and secretory signals that are at least about 80% identical to SEQ ID NO: 5 or a fragment thereof of at least 15 amino acids,

3) -SEQ ID NO:6, fragments thereof of at least 15 amino acids, signal encoded by a nucleic acid that hybridizes to a nucleic acid encoding SEQ ID NO: 6 or a fragment thereof of at least 15 amino acids, and secretory signals that are at least about 80% identical to SEQ ID NO: 6 or a fragment thereof of at least 15 amino acids,

Vectors encoding species of polypeptides of interest selected from the group consisting of a receptor, an antibody fragment, galanin, neuropeptide Y, cholecystokinin, thyrotropin-releasing hormone, neurotensin, oxytocin, acidic fibroblast growth factor, basic fibroblast growth factor, nerve growth factor, glial cell derived growth factor, met-enkephalin, leu-enkephalin, dynorphin, beta-endorphin, leptin, a semaphorin peptide, tyrosine hydroxylase, aromatic amino acid decarboxylase, brain-derived neurotrophic factor, nerve growth factor, superoxide dismutase, catalase, glutathione peroxidase, adenosine A-1 receptor, GABA-A receptor, glutamate decarboxylase, somatostatin, and an antitumor agent.

Target cells or delivery regions selected from the group consisting of a neuron, astrocyte, oligodendrocyte, microglial cell, fibroblast, endothelial cell, astroglial cell, or ependymal cell, and a cell from the limbic system, spinal cord, neocodex, thalamus, hypothalamus, epithalamaus, pineal gland, corpus striatum, cerebrum, basal ganglia, amygdala, brainstem, cerebrum, cerebellum, striatum, hippocampus, inferior colliculus, pituitary or substantia nigra.

The claims are deemed to correspond to the species listed above in the following manner:

Claim 6 corresponds to the species of secretory signals. Claims 1-5, 7-14, 18-20, and 24-31 are generic.

Claims 11-14, correspond to the species of polypeptides of interest. Claims 1-10, and 15-40 are generic.

Claims 20, 23, 31, and 40 correspond to the species of target cells and delivery regions. Claims 18, 19, 21, 22, 24-30, and 32-39 are generic.

The total number of inventions was arrived at by determining the number of combinations of species i.e. 3 species of secretory signal X 30 species of polypeptide X 27 species of cells or delivery regions X 4 groups.